EXHIBIT 3

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Effect of Caffeine and 1,3-Dimethylamylamine on Exercise Performance and Blood Markers of Lipolysis and Oxidative Stress in Trained Men and Women

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Background: Caffeine is one of the most widely used ergogenic aids worldwide. Recently, caffeine has been combined with 1,3-dimethylamylamine (1,3-D) in an attempt to improve exercise performance and related variables. We investigated the effect of caffeine and 1,3-D alone and in combination on exercise performance and blood markers of lipolysis and oxidative stress.
Methods: Twelve exercise-trained subjects ingested placebo, caffeine ($4\ mg \cdot kg^{-1}$), 1,3-D ($1\ mg \cdot kg^{-1}$), or caffeine + 1,3-D, 60 minutes before completing a 10 km run. Blood was collected before intake, immediately pre-exercise, and at 5 and 30 minutes postexercise. Samples were analyzed for glycerol, free fatty acids (FFAs), malondialdehyde, nitrate/nitrite, and trolox equivalent antioxidant capacity (TEAC).
Results: Run time (minutes) was not different for placebo (52.55 ± 1.96), caffeine (52.00 ± 1.88), 1,3-D (52.02 ± 1.86), or caffeine + 1,3-D (52.46 ± 1.94) ($p > 0.05$). Glycerol and FFA were higher 5 and 30 minutes postexercise compared with pretreatment and pre-exercise ($p < 0.05$). A condition effect was noted for glycerol ($p = 0.01$), with higher values for 1,3-D compared with caffeine + 1,3-D ($p < 0.05$). A condition effect was noted for TEAC ($p = 0.0001$), with higher values for placebo compared with caffeine and caffeine + 1,3-D, and higher values for 1,3-D compared with caffeine ($p < 0.05$). No other effects were noted for any measured variable ($p > 0.05$).
Conclusion: We report for the first time that caffeine + 1,3D does not improve exercise performance as measured by run time. Isolated ingestion of 1,3-D results in the greatest increase in postexercise glycerol and FFA. Caffeine or 1,3-D alone or in combination does not differently affect oxidative stress biomarkers.

Introduction

Caffeine is arguably one of the most widely used ergogenic aids in the world[1]—included within hundreds of different encapsulated, pilled, and powdered dietary supplements. One recent addition to the ergogenic supplement category is a component of the Pelargonium graveolens plant (1,3-dimethylamylamine [1,3-D]),[2] which has anecdotally been noted to possess a powerful stimulatory effect. The combination of caffeine and 1,3-D is becoming increasingly popular among athletes due to the potential for performance enhancement, as well as related effects. Specifically, caffeine has been described as a lipolytic agent,[3–5] allowing for the release of free fatty acids (FFAs) from storage depots. However, data pertaining to the lipolytic effects of caffeine are mixed, with some studies reporting no increase in lipolysis after caffeine ingestion[6,7] or a derivative of caffeine.[8] The literature pertaining to the use of 1,3-D is scant; however, this agent is a simple aliphatic amine that functions as a norepinephrine reuptake inhibitor and/or norepinephrine releasing agent.[9] Based on these effects, 1,3-D may lead to enhanced hormone sensitive lipase activity, ultimately allowing for the release of FFA to undergo oxidation for energy production purposes.[10]

Apart from the potential lipolytic effects of these agents, both caffeine and 1,3-D appear to enhance focus and attention during exercise. Although scientific evidence is available to support this effect for caffeine,[11,12] only anecdotal reports are available for 1,3-D. Considering what has been just stated, the combination of these two agents may aid exercise performance due to a potential lipolytic effect, thus allowing for increased FFA to be available as a fuel source during moderate duration exercise, potentially sparing muscle glycogen, while also enhancing focus during training.

Beyond what has been just stated, evidence from nonexercising humans indicates that caffeine may provide antioxidant benefits,[13,14] thus potentially attenuating oxidative stress. This antioxidant effect has also been noted for 1,3-D.[15] The improvement in antioxidant status may be attractive to exercise enthusiasts, as increased oxidative stress is often noted in response to strenuous physical work.[16] It is possible that the use of caffeine and 1,3-D alone or in combination may serve to minimize the oxidative stress resulting from an

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acute bout of strenuous exercise. To date, no studies have determined this.

Although both 1,3-D and caffeine are known to acutely elevate blood pressure,[17] caffeine has also been noted to induce vasorelaxation by stimulating the production of nitric oxide.[18] Collectively considering the above, we investigated the effect of caffeine and 1,3-D on exercise performance and blood markers of lipolysis and oxidative stress in a sample of exercise-trained men and women. We also measured plasma nitrate/nitrite (NOx) and hemodynamic variables before and after treatment with these agents.

Materials and Methods

Subjects

Young and healthy, exercise-trained men ($n=6$) and women ($n=6$) participated in this investigation. All subjects completed a medical history and physical activity questionnaire to determine eligibility for participation. No subject was a smoker or had diagnosed cardiovascular (e.g., hypertension) or metabolic disease. All subjects were regular runners. Three subjects reported using caffeine daily, through consumption of coffee or espresso ($n=2$), or green tea ($n=1$). The mean daily caffeine intake for these subjects was 230 mg·day^{-1}. Three subjects reported using coffee infrequently throughout the week. Subjects' heart rate and blood pressure, height, weight, waist and hip circumference, and skinfold thickness (seven site) for estimation of body fat percentage were measured and used to describe the subjects. These descriptive characteristics, including exercise training history, are presented in Table 1. After screening procedures, the subjects performed a 3 km familiarization run on an outdoor track—that used for the experimental test days. All experimental procedures were performed in accordance with the Helsinki Declaration. The University of Memphis Human Subjects Committee approved all experimental procedures (H10-49), and subjects provided verbal and written consent before participating in this study.

Testing

All testing procedures described next were identical for all four test days. Subjects reported to the laboratory in a 10 hours fasted state, and all testing was completed in the morning hours (0500–0900). The time of testing was matched for subjects for all four conditions. Subjects were instructed not to exercise for the 24 hours before each test day. On arrival to the laboratory, the subjects were asked to void and then rested quietly for 10 minutes in a seated position. After this, the subjects' heart rate (HR: via radial artery palpation for 60 seconds by two trained technicians) and systolic (SBP) and diastolic (DBP) blood pressure (via auscultation using a dual earpiece stethoscope) were measured. Rate pressure product (RPP) was calculated as an indication of myocardial work by using the equation: HR×SBP. A blood sample was then collected. Subjects were then provided their assigned condition and ingested this in the presence of an investigator. After the ingestion of the assigned condition, the subjects rested for 60 minutes and then began the exercise bout. Before starting the exercise bout, HR and blood pressure were measured, and a blood sample was collected. This same collection (HR, blood pressure, and blood) was repeated at 5 and 30 minutes postexercise. Subjects consumed no food during the entire testing period; however, water was allowed *ad libitum* and matched for subjects on the days of testing.

The exercise bout consisted of a 10 km run on an outdoor track. For all bouts, subjects were encouraged to complete the run as quickly as possible. HR was monitored by using a HR monitor. The Borg (6–20) scale of exertion was used to allow subjects to indicate their level of perceived work. Subjects were also asked to rate their overall mood/vigor by using a 0–10 point scale. HR, perceived exertion, and mood/vigor were recorded at the end of each 2 km period. At the end of exercise, run time was recorded, and the subjects returned to the lab for the postexercise measures. It should be noted that the walk from the track to the lab is estimated to be <150 feet. Test days were separated by 1 week. The environmental conditions for each test day were recorded and noted to be very good for running. For example, starting run temperatures ranged from 44°F to 68°F, with mostly clear skies on all test days and wind noted only on three of the 18 days on which the subjects ran.

Table 1. Characteristics of 12 Exercise-Trained Subjects

Variable	*Value*
Age (years)	21.9±2.9
Height (cm)	173.2±10.7
Weight (kg)	67.6±11.9
Body mass index (kg·m^{-2})	22.4±2.5
Body fat (%)	15.6±7.8
Waist (cm)	72.7±6.8
Hip (cm)	96.8±4.8
Waist:Hip	0.75±0.04
Years of anerobic exercise training	4.5±2.8
Hours per week of anerobic exercise	3.0±1.6
Years of aerobic exercise training	5.2±3.7
Hours per week of aerobic exercise	6.3±4.3

Data are mean±standard deviation.

Conditions

Conditions were received in a random order by using a double-blind design: placebo (30 g of carbohydrate); caffeine (30 g of carbohydrate + caffeine at 4 mg·kg body mass^{-1}); 1,3-D (30 g of carbohydrate + 1,3-D at 1 mg·kg body mass^{-1}); or caffeine + 1,3-D (30 g of carbohydrate + caffeine at 4 mg·kg body mass^{-1} and 1,3-D at 1 mg·kg body mass^{-1}). The dosage of caffeine used in the current design was based on previous studies using caffeine for an ergogenic benefit—in particular, one study using caffeine at a dosage of 3 mg·kg body mass^{-1} and noting an improvement in 8 km run performance.[19] However, it should be noted that many studies have used caffeine at a dosage >4 mg·kg body mass^{-1}.[20–23] Our use of a relatively low dosage of caffeine was based on our inclusion of 1,3-D in the current design, and our concern over excessive stimulation with higher dosing. The dosage of 1,3-D was based on anecdotal reports of individuals using this ingredient, coupled with a review of nutritional panels of dietary supplements containing this ingredient.

The 1,3-D (1,3-dimethylamylamine HCL) was purchased from Waseta International (Shanghia, China), and the caffeine (caffeine anhydrous) was purchased from Hi Tech

Table 2. Dietary Data of Exercise-Trained Subjects Receiving Placebo, Caffeine, 1,3-Dimethylamylamine, or Caffeine + 1,3-Dimethylamylamine

Variable	*Placebo*	*Caffeine*	*1,3-Dimethylamylamine*	*Caffeine + 1,3-dimethylamylamine*
Kilocalories	2358 ± 209	2083 ± 212	2294 ± 247	2070 ± 238
Protein (g)	104 ± 14	100 ± 13	109 ± 17	97 ± 17
Carbohydrate (g)	329 ± 38	331 ± 44	320 ± 45	283 ± 42
Fat (g)	77 ± 8	74 ± 11	69 ± 10	66 ± 10
Vitamin C (mg)	90 ± 31	90 ± 28	96 ± 25	81 ± 21
Vitamin E (mg)	7 ± 4	12 ± 5	9 ± 3	7 ± 2
Vitamin A (RE)	417 ± 146	551 ± 164	643 ± 191	463 ± 135

Data are mean ± SEM. No statistical difference was noted between conditions in kilocalories ($p = 0.94$), protein ($p = 0.76$), carbohydrate ($p = 0.91$), fat ($p = 0.44$), vitamin C ($p = 0.99$), vitamin E ($p = 0.77$), or vitamin A ($p = 0.92$).
RE, retinol equivalents; SEM, standard error of the mean.

Pharmaceuticals, Inc. (Norcross, GA). Certificates of analysis for each ingredient indicated purity. All conditions were mixed into 500 mL of water and, the beverage was fruit-punch flavored. The carbohydrate source was maltodextrin, and our dosage of 30 g was selected to provide 120 kcal, an amount similar to what is provided within many commonly consumed sport drinks.

Blood collection and biochemistry

A total of four venous blood samples were taken from subjects' forearm veins via needle and Vacutainer®. Blood for collection of serum was allowed to clot for 30 minutes at room temperature and then processed in a refrigerated centrifuge (4°C for 15 minutes at 1500*g*). Blood for collection of plasma was immediately processed in a refrigerated centrifuge (4°C for 15 minutes at 1500*g*). Samples were stored in multiple aliquots at −70°C. Glycerol was analyzed in plasma by using the Free Glycerol Determination Kit (FG0100) and Glycerol Standard (G7793), following the manufacturer's instructions (Sigma Aldrich). FFAs were analyzed in plasma by using the Free Fatty Acid Quantification Kit (K612-100), following the manufacturer's instructions (BioVision). As a measure of lipid peroxidation, malondialdehyde (MDA) was analyzed in plasma by following the procedures of Jentzsch *et al.* using.[24] reagents purchased from Northwest Life Science Specialties (Vancouver, WA). As a surrogate measure of nitric oxide, NOx was analyzed in plasma by using a commercially available colorimetric assay kit (Cayman Chemical, Ann Arbor, MI) according to the procedures provided by the manufacturer. Antioxidant capacity was analyzed in serum by using the trolox equivalent antioxidant capacity (TEAC) assay using procedures outlined by the reagent provider (Sigma Chemical, St. Louis, MO). All samples were analyzed on first thaw.

Dietary records

All subjects were instructed to maintain their normal diet during the study period and to record all food and drink consumed during the 24 hours before each test day. Records were reviewed with each subject for accuracy and then analyzed using Food Processor SQL, version 9.9 (ESHA Research, Salem, OR).

Statistical analysis

Exercise performance and dietary data were analyzed by using an analysis of variance (ANOVA). All other data were analyzed by using a 4 (condition) × 4 (time) ANOVA. Tukey *post hoc* tests were performed when necessary. Statistical significance was set at $p \leq 0.05$. The analyses were done using JMP statistical software (version 4.0.3, SAS Institute, Cary, NC). Data are presented as mean ± standard error of the mean, except for subject characteristics that are presented as mean ± standard deviation.

Results

All subjects successfully completed all test days. The conditions were generally well-tolerated; however, the combination of caffeine + 1,3-D resulted in extreme feelings of euphoria in many subjects, some of whom claimed that their exercise performance may have been better if not receiving the condition. Dietary data during the day before each test day were not different between conditions ($p > 0.05$). Data are presented in Table 2.

Performance data

With regard to the performance data, run time ($p = 0.99$), perceived exertion ($p = 0.71$), mood/vigor ($p = 0.41$), and HR ($p = 0.91$) were not different between conditions. Data are presented in Table 3.

Table 3. Run Time, Perceived Exertion, Mood/Vigor, and Heart Rate of Exercise-Trained Subjects Receiving Placebo, Caffeine, 1,3-Dimethylamylamine, or Caffeine + 1,3-dimethylamylamine

Variable	*Placebo*	*Caffeine*	*1,3-Dimethylamylamine*	*Caffeine + 1,3-dimethylamylamine*
Run time (minutes)	52.55 ± 1.96	52.00 ± 1.88	52.02 ± 1.86	52.46 ± 1.94
Perceived exertion (6–20 scale)	13.70 ± 0.47	14.17 ± 0.36	13.93 ± 0.65	14.46 ± 0.40
Mood/vigor (0–10 scale)	5.02 ± 0.31	5.10 ± 0.41	5.57 ± 0.50	4.58 ± 0.39
Heart rate (bpm)	180.61 ± 3.23	178.53 ± 3.94	181.83 ± 2.84	179.43 ± 3.62

Data are mean ± SEM. No statistical difference was noted between conditions in run time ($p = 0.99$), perceived exertion ($p = 0.71$), mood/vigor ($p = 0.41$), or heart rate ($p = 0.91$). Perceived exertion, mood/vigor, and heart rate were recorded every 2 km. Data were averaged across all times for each subject, and mean data are presented in the table.

Biochemical data

With regard to the biochemical data, results were as follows. For glycerol, a condition effect was noted ($p=0.01$), with 1,3-D greater than caffeine + 1,3-D ($p<0.05$). A time effect was noted ($p<0.0001$), with 5 minutes postexercise and 30 minutes postexercise greater than pretreatment and pre-exercise; 5 minutes postexercise >30 minutes postexercise ($p<0.05$). No condition × time interaction was noted ($p=0.23$); however, values were highest for 1,3-D and lowest for caffeine + 1,3-D at the 5 and 30 minutes postexercise times. For FFA, no condition effect was noted ($p=0.06$). A time effect was noted ($p<0.0001$), with 5 minutes postexercise and 30 minutes postexercise greater than pretreatment and pre-exercise; pretreatment greater than pre-exercise; and 5 minutes postexercise >30 minutes postexercise ($p<0.05$). No condition × time interaction was noted ($p=0.14$); however, values were highest for 1,3-D at the 5 and 30 minutes postexercise times. Data for glycerol (A) and FFA (B) are presented in Figure 1.

For MDA, no condition ($p=0.84$), time ($p=0.83$), or condition × time interaction effect was noted ($p=0.98$). For NOx, no condition ($p=0.16$), time ($p=0.91$), or condition × time interaction effect was noted ($p=0.99$). For TEAC, a condition effect was noted ($p=0.0001$), with placebo greater than caffeine and caffeine + 1,3-D; 1,3-D greater than caffeine ($p<0.05$). No time ($p=0.10$) or condition × time interaction effect was noted ($p=0.96$). Data for MDA (A), NOx (B), and TEAC (C) are presented in Figure 2.

Hemodynamic data

With regard to the hemodynamic data, results were as follows. For HR, a condition effect was noted ($p=0.02$), with caffeine + 1,3-D greater than 1,3-D ($p<0.05$). A time effect was noted ($p<0.0001$), with 5 minutes postexercise and 30 minutes postexercise greater than pretreatment and pre-exercise ($p<0.05$). No condition × time interaction was noted ($p=0.94$). For SBP, a condition effect was noted ($p<0.0001$), with caffeine and 1,3-D greater than placebo and caffeine + 1,3-D ($p<0.05$). A time effect was noted ($p<0.0001$), with pre-exercise and 5 minutes postexercise greater than pretreatment and 30 minutes postexercise ($p<0.05$). A condition × time interaction was also noted ($p<0.0001$). For DBP, a condition effect was noted ($p=0.03$), with 1,3-D greater than caffeine + 1,3-D ($p<0.05$). A time effect was noted ($p<0.0001$), with pre-exercise greater than all other times ($p<0.05$). No condition × time interaction was noted ($p=0.23$). For RPP, no condition effect was noted ($p=0.33$). A time effect was noted ($p<0.0001$), with pretreatment less than all other times; 5 minutes postexercise and 30 minutes postexercise greater than pre-exercise; and 5 minutes postexercise >30 minutes postexercise ($p<0.05$). No condition × time interaction was noted ($p=0.95$). Data are presented in Table 4.

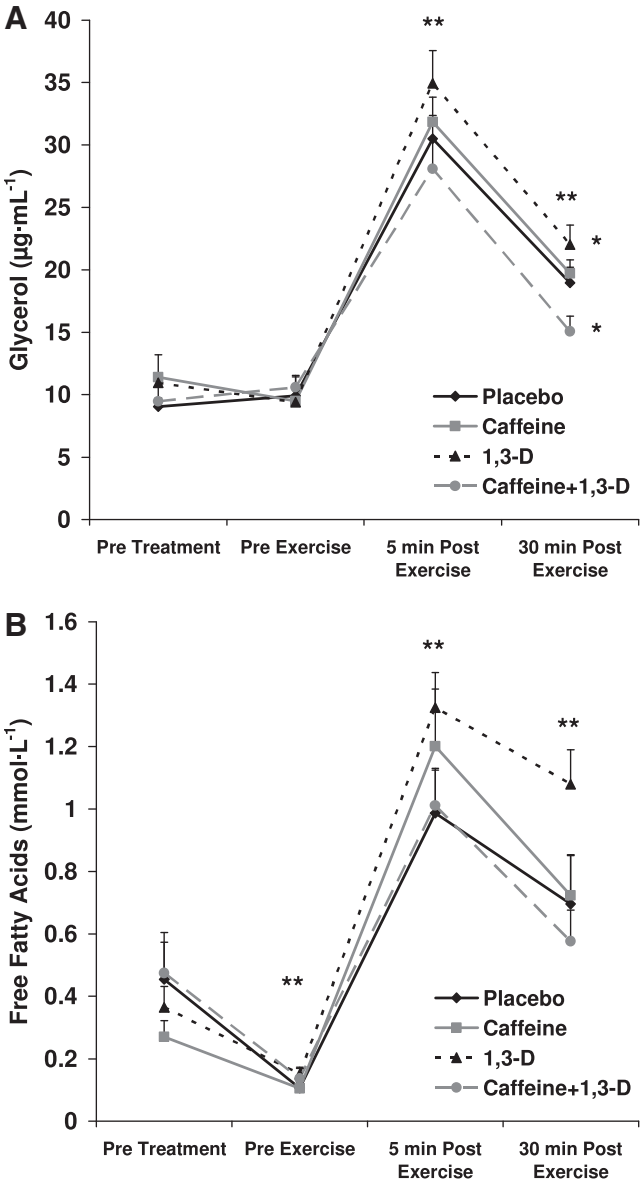
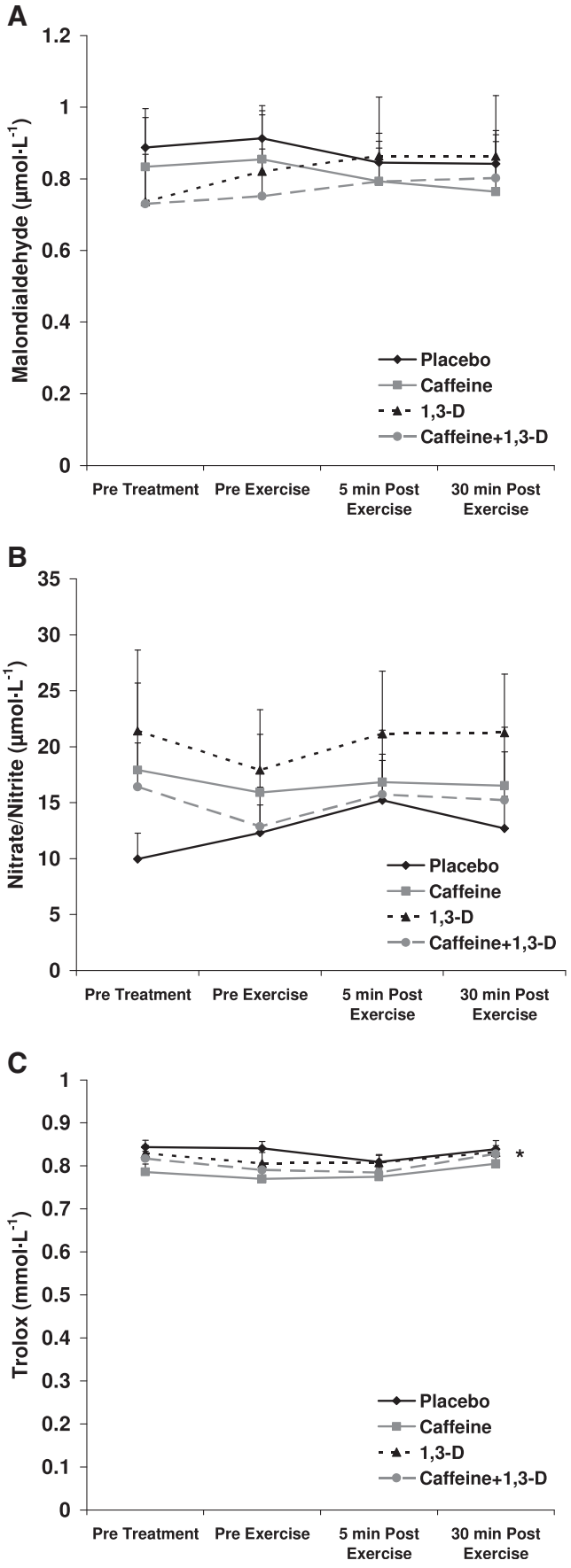


FIG. 1. Plasma glycerol **(A)** and free fatty acids **(B)** of exercise-trained subjects receiving placebo, caffeine, 1,3-dimethylamylamine, or caffeine + 1,3-dimethylamylamine. Glycerol: *A condition effect was noted ($p=0.01$); 1,3-D > caffeine + 1,3-D ($p<0.05$). **A time effect was noted ($p<0.0001$); 5 minutes postexercise and 30 minutes postexercise > pretreatment and pre-exercise; 5 minutes postexercise > 30 minutes postexercise ($p<0.05$). No condition × time effect was noted ($p=0.23$). Free fatty acids: No condition effect was noted ($p=0.06$). **A time effect was noted ($p<0.0001$); 5 minutes postexercise and 30 minutes postexercise > pretreatment and pre-exercise; pretreatment > pre-exercise; and 5 minutes postexercise > 30 minutes postexercise ($p<0.05$). No condition × time effect was noted ($p=0.14$).

Discussion

Data from the current investigation indicate that (1) ingestion of caffeine or 1,3-D alone or in combination does not improve exercise performance as measured by run time; (2) ingestion of 1,3-D results in the greatest increase in postexercise glycerol and FFA concentrations; (3) caffeine or 1,3-D alone or in combination does not differently effect oxidative stress biomarkers pre- or postexercise; and (4) caffeine and 1,3-D alone increase SBP. This is the first study to investigate the effect of 1,3-D alone and in combination with caffeine on exercise performance and related variables.



Our outcome of greatest interest in this investigation was that of exercise performance (run time). We noted no statistically significant effect of condition on run time, although times for both caffeine and 1,3-D were approximately 30 seconds lower than for placebo (Table 3). Although not statistically different, it is possible that such a subtle difference in run time might prove beneficial in competition. It is also possible that these ingredients may benefit performance during other exercise challenges (e.g., resistance exercise). Additional work is needed to determine this.

The combination of caffeine + 1,3-D did not result in as favorable of an outcome with regard to run time as compared with the two agents alone. We believe that the dosage of each agent was too high when delivered in combination, as many subjects reported feeling too euphoric before and during the exercise bout. Perhaps a lower dosage would have yielded more positive results. Our findings of no significant improvement in exercise performance for caffeine compared with placebo agree with several reports[25–30]; however, they are in conflict with others.[3,19–23,31–34] Multiple factors are likely involved in the discrepancy across studies, including caffeine dosage, the combination of caffeine with other dietary nutrients and stimulants, the inclusion of a pre-exercise meal, the type of exercise test being performed, the time of day of the exercise test relative to subjects' usual exercise time, where the exercise test is being performed, climatic temperature and conditions (assuming outdoors), and training status of subjects.

Beyond the measure of run time, no other performance-related measure was different between conditions (perceived exertion, mood/vigor, and HR). In support of subjects' comments, the combination of caffeine + 1,3-D yielded the highest perceived exertion and the lowest mood/vigor. Again, it is likely that the combined dosage of these two agents was simply too high, and subjects did not tolerate this condition well. Future work should seek to determine whether a lower dosage of each agent would result in a more favorable response—as has been frequently cited in anecdotal reports using a combination of these two agents. In agreement with the pre- and postexercise measure of HR, the exercise HR data were not different for caffeine or 1,3-D alone or in combination, as compared with placebo. These findings confirm that the treatment dosages used in the current study do not cause an exercise-induced exacerbation in HR.

As expected, we noted an increase in both glycerol and FFA in response to exercise (Fig. 1). These findings are well supported by the exercise literature, in particular with regard

FIG. 2. Plasma malondialdehyde **(A)**, nitrate/nitrite **(B)**, and trolox equivalent antioxidant capacity **(C)** of exercise-trained subjects receiving placebo, caffeine, 1,3-dimethylamylamine, or caffeine + 1,3-dimethylamylamine. Malondialdehyde: No condition effect was noted ($p = 0.84$). No time effect was noted ($p = 0.83$). No condition × time effect was noted ($p = 0.98$). nitrate/nitrite: No condition effect was noted ($p = 0.16$). No time effect was noted ($p = 0.91$). No condition × time effect was noted ($p = 0.99$). Trolox equivalent antioxidant capacity: *A condition effect was noted ($p = 0.0001$); placebo > caffeine and caffeine + 1,3-D; 1,3-D > caffeine ($p < 0.05$); No time effect was noted ($p = 0.10$). No condition × time effect was noted ($p = 0.96$).

Table 4. Hemodynamic Data of Exercise-Trained Subjects Receiving Placebo, Caffeine, 1,3-Dimethylamylamine, or Caffeine + 1,3-dimethylamylamine

Variable	*Heart rate (bpm)*	*Systolic blood pressure (mmHg)*	*Diastolic blood pressure (mmHg)*	*Rate pressure product*
Placebo Pretreatment	57 ± 3	113 ± 1	64 ± 3	6394 ± 289
Placebo Pre-exercise	60 ± 2	121 ± 3	67 ± 3	7202 ± 286
Placebo 5 minutes postexercise	109 ± 3	126 ± 3	64 ± 2	13,711 ± 505
Placebo 30 minutes postexercise	85 ± 4	112 ± 3	67 ± 2	9543 ± 532
Caffeine Pretreatment	59 ± 2	113 ± 2	65 ± 2	6670 ± 259
Caffeine Pre-exercise	59 ± 2	140 ± 3	75 ± 5	8265 ± 339
Caffeine 5 minutes postexercise	104 ± 6	141 ± 4	65 ± 3	14,596 ± 939
Caffeine 30 minutes postexercise	79 ± 5	125 ± 4	65 ± 3	9913 ± 696
1,3-D Pretreatment	55 ± 2	113 ± 3	66 ± 2	6175 ± 247
1,3-D Pre-exercise	53 ± 2	150 ± 5	81 ± 3	8033 ± 420
1,3-D 5 minutes postexercise	100 ± 5	147 ± 4	66 ± 3	14,787 ± 944
1,3-D 30 minutes postexercise	78 ± 4	128 ± 4	69 ± 3	10,014 ± 725
Caffeine + 1,3-D Pretreatment	58 ± 3	117 ± 3	63 ± 2	6845 ± 417
Caffeine + 1,3-D Pre-exercise	62 ± 3	120 ± 2	67 ± 3	7351 ± 340
Caffeine + 1,3-D 5 minutes postexercise	112 ± 5	126 ± 3	61 ± 2	14,181 ± 780
Caffeine + 1,3-D 30 minutes postexercise	83 ± 3	111 ± 3	69 ± 3	9256 ± 438

Data are mean ± SEM. *Heart rate*: Condition: $p = 0.02$; caffeine + 1,3-D > 1,3-D ($p < 0.05$). Time: $p < 0.0001$; 5 minutes postexercise and 30 minutes postexercise > pretreatment and pre-exercise ($p < 0.05$). Condition × Time: $p = 0.94$. *Systolic blood pressure*: Condition: $p < 0.0001$; caffeine and 1,3-D > placebo and caffeine + 1,3-D ($p < 0.05$). Time: $p < 0.0001$; pre-exercise and 5 minutes postexercise > pre-treatment and 30 minutes postexercise ($p < 0.05$). Condition × Time: $p < 0.0001$. Diastolic blood pressure: Condition: $p = 0.03$; 1,3-D > caffeine + 1,3-D ($p < 0.05$). Time: $p < 0.0001$; pre-exercise > all other times ($p < 0.05$). Condition × Time: $p = 0.23$. *Rate pressure product*: Condition: $p = 0.33$. Time: $p < 0.0001$; pretreatment < all other times; 5 minutes postexercise and 30 minutes postexercise > pre-exercise; 5 minutes postexercise > 30 minutes postexercise ($p < 0.05$). Condition × Time: $p = 0.95$.

1,3-D, 1,3-dimethylamylamine.

to aerobic exercise[23,32,35] However, we found no interaction effects for either glycerol or FFA, with a relatively similar response curve noted for all conditions. Some studies using caffeine as a lipolytic agent have noted increased lipolysis at rest[36,37] and with exercise.[23,31,32] However, others have not,[25,26,35,38,39] essentially corroborating our findings or little difference between the caffeine and placebo conditions either pre- or postexercise.

A condition effect was noted for glycerol, with 1,3-D exhibiting the greatest increase postexercise—statistically higher than the combination of caffeine + 1,3-D. The same general finding was noted for FFA, with 1,3-D demonstrating the highest overall values; however, the condition effect failed to reach significance ($p = 0.06$). Interestingly, the combination of caffeine + 1,3-D demonstrated the lowest values for both glycerol and FFA, which somewhat helps explain the low-performance-related findings. Based on the assumed lipolytic properties of both caffeine and 1,3-D, we do not have a clear explanation as to why both glycerol and FFA concentrations were lowest in the combined condition—although this may be related to the hormonal response to treatment. For example, if a hormone such as insulin was elevated to a greater extent with the combined treatment, then this may help explain our findings for lower FFA and glycerol, as elevations in insulin[40] are known to suppress fatty acid release and oxidation. Of course, we have no evidence to support this hypothesis, in particular when considering that greater insulin release is not observed with caffeine intake alone.[41] Clearly, neither caffeine or 1,3-D alone resulted in an impairment in FFA and glycerol release (Fig. 1). Therefore, if our hypothesis just stated were confirmed, then there would need to be some synergy between the two treatments to promote this suppression in response.

The increase in glycerol and FFA with 1,3-D may be supported by the physiological effect of this agent as a norepinephrine releasing agent or norepinephrine reuptake

inhibitor. It is well described that norepinephrine has an effect on lipolysis,[42] which seems to occur via activation of hormone sensitive lipase, as well as through hormone sensitive lipase translocation from the cytosol to the lipid droplets within fat cells.[10] As just discussed, the reason that the addition of caffeine to the 1,3-D did not enhance this effect, but rather inhibited this effect, remains to be determined.

Despite some literature suggesting a potential antioxidant effect of caffeine[13,14] and 1,3-D,[15] we did not observe anything different in either MDA or NOx between conditions pre- or postexercise. However, it should be noted that the exercise itself did not result in an increase in these measures, thus making it difficult to detect any potential antioxidant benefit of either agent. As has been clearly presented in the literature, exercise has the potential to induce an oxidative stress, but this certainly does not occur in all circumstances.[16] In fact, many studies including exercise-trained subjects fail to note a significant increase in oxidative stress biomarkers. This was the case in the current investigation, despite the performance of a 10 km performance run. Our findings highlight the adaptive nature of the human body, with our exercise-trained subjects experiencing very little oxidative stress in a manner consistent with the principle of hormesis.[43]

Although no condition differences were noted for MDA or NOx, a condition effect was noted for TEAC, with the lowest values observed during the caffeine condition. However, it should be noted that the condition effect for TEAC appeared to be most influenced by pretreatment TEAC values (which were highest for the placebo condition and lowest for the caffeine condition), rather than by TEAC in response to treatment and exercise. In fact, the overall response curve for TEAC was very similar for all conditions (Fig. 2C). Although a decrease in TEAC may indicate an oxidative stress, considering the relatively minor change in this variable in response to exercise for all conditions, from a physiological point of view, we do not believe that the subtle condition differences noted for TEAC have significant meaning. This is underscored by the very similar TEAC values observed for all conditions at the 30 minute postexercise time, which were similar to or greater than pretreatment values for all conditions.

As expected, we noted an increase in all measured hemodynamic variables from pre- to postexercise. In addition, we noted a higher SBP with both the caffeine and 1,3-D conditions compared with placebo. Interestingly, the combination of caffeine + 1,3-D did not result in a higher SBP compared with placebo. In fact, the response was nearly identical to that of placebo—for both SBP and HR. At present, we admit that we have no explanation for these findings, with the possible exception of the following: Although 1,3-D is thought to promote vasoconstriction, caffeine may act to induce vasorelaxation.[18] It is possible that the combination of the two agents produced an acute vasorelaxation effect to allow for blood pressure to be reduced (in particular, considering that HR remained elevated above that for caffeine or 1,3-D alone). Future study is needed to further determine the independent and combined effects of these two agents on HR and blood pressure.

Although not identical in structure, it has been suggested that 1,3-D may have similar functional properties as ephedrine, which has been noted to exhibit mixed results in terms of HR and blood pressure, with the majority of work indicating a moderate increase in these variables.[44–47] Our previous work with 1,3-D and caffeine alone, while subjects remained at rest, have noted similar findings for SBP.[17] However, we noted an additive effect of the two agents when combined. This is in opposition to the findings of the current study, which obviously incorporated exercise into the design. Perhaps the alteration in vascular tone as a result of an acute exercise bout impacts the overall hemodynamic effects of subjects using a combination of caffeine + 1,3-D.

Finally, it should be reiterated that subjects were tested in a 10 hour fasted state, with the exception of the 30 g of maltodextrin provided within the conditions. It is possible that the hemodynamic effects could be attenuated if the conditions were ingested in a fed state, as may be typical for individuals using dietary supplements such as caffeine and 1,3-D. Moreover, the ingestion of a small standardized meal and/or the performance of testing later in the day may have allowed for better exercise performance, as such conditions may better mimic subjects' usual exercise routine. Of course, by incorporating this into the design, more variability would be introduced into the experiment.

Conclusions

We report for the first time that acute oral ingestion of caffeine and 1,3-D alone or in combination does not significantly improve exercise performance as measured by run time. Further, ingestion of either 1,3-D or caffeine alone results in the greatest increase in postexercise glycerol and FFA concentrations, whereas the combination of the two agents results in concentrations that are more similar to placebo. Neither agent has an impact on oxidative stress biomarkers, either before or after exercise. Finally, caffeine or 1,3-D alone increases SBP, without adversely impacting other hemodynamic variables. Future studies are needed using different dosages of these agents, as well as different exercise tests—possibly at a time of day more similar to subjects' usual training time, to determine whether more favorable effects can be observed after acute intake of caffeine or 1,3-D alone and in combination.

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